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Biostimulant Application Alleviates the Negative Effects of Deficit Irrigation and Improves Growth Performance, Essential Oil Yield and Water-Use Efficiency of Mint Crop

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Abstract: The scarcity of water is limiting crop production and is one of the most important stressors that severely affects crop yield, and it may also decrease the quality of the final products. Most of the medicinal and aromatic plants are considered resilient to water stress and constitute a sustainable choice for crop production in arid and semiarid conditions. In the present study, we examined the effect of scheduled deficit irrigation (e.g., I1: 40% of field capacity); I2: 70% of field capacity; and I3: 100% of field capacity) combined with biostimulant application (four different products that consisted of nitrogenous compounds and carboxylic acids (M1); nitrogenous compounds and seaweed extracts (M2); humic and fulvic acids and seaweed extracts (M3); and CaO, SiO_2, calcium mobilization and translocation factor and microminerals (M4)) on crop performance and essential oil production of mint plants (*Mentha arvensis* L.). Our aim was to define an irrigation regime that increases water-use efficiency and the biostimulant products that alleviate water stress effects. Our results indicate that moderate deficit irrigation (I2 treatment) and biostimulants that contained seaweed extracts and nitrogenous compounds and humic and fulvic acids (M2 and M3 treatments, respectively) significantly improved yield parameters in terms of fresh and dry herb yield and essential oil production. Moreover, the same biostimulant treatments significantly increased water-use efficiency of mint crops based on the various yield parameters tested in this study. In conclusion, our results indicate that selection of proper biostimulatory products may allow to apply deficit irrigation regimes in mint cultivation without compromising the crop performance in terms of both biomass production and essential oil yield. Therefore, the combination of these agronomic tools could facilitate water saving strategies in arid and semiarid regions and contribute to the sustainable management of water resources.

Keywords: medicinal and aromatic plants; *Mentha arvensis* L.; water stress; seaweed extracts; water-use efficiency; dry herb yield

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1. Introduction

Mentha arvensis L., often called Japanese mint, field mint, wild mint or corn mint, is grown commercially for the production of essential oil, which is widely used in aromatherapy, as well as in the food and pharmaceutical industries [1–6]. Considering its agronomic requirements, mint is a species with shallow roots that needs large amounts of water; therefore, water shortage might significantly lower its productivity [7]. The most important source of natural mint oil that is used as raw material in the pharmaceutical and fragrance industries is *Mentha arvensis* L., with a chemical composition that varies depending on the species and variability in agro-climatic conditions [8]. The need for more affordable and environmentally friendly production methods for this crop is driven by the ongoing rise in demand and cost of mint oil. Moreover, the market for mint oil has grown rapidly during the last years due to the increasing demand for pure menthol around the world [9].

Aromatic and medicinal plants may be readily included into organic agriculture using biostimulants and organic fertilizers while still producing sufficient amounts of biomass and essential oils due to their minimal input requirements [10]. The two most important agronomical inputs to ensure the best crop quality and performance relate to irrigation and fertilizer application [11,12]. In particular, in order to achieve optimal growth and high yields, wild mint needs frequent irrigation during the summer, especially when it is planted as a summer crop [13,14]. Ram et al. [15] and Singh et al. [16,17] claimed that irrigation has a beneficial effect on raising the yields of herbs and essential oils for a variety of mint species and fragrant grasses, while Akbarzadeh et al. [18] suggested that partial root-zone drying was more effective in increasing water productivity than regulated deficit irrigation in terms of essential oil production. In this context, the application of water management strategies as deficit irrigation could allow to increase the water-use efficiency of medicinal and aromatic crops without compromising the herb and essential oil yield and quality [19,20].

Unfortunately, irrigation water of high quality is becoming more and more scarce due to anthropogenic activities that deteriorate water reservoirs or limit the overall availability, thus severely affecting crop production and the quality of products [21,22]. Since the world's population is expected to double by 2050, the demand for food will increase, making necessary more efficient use of the available productive land and high-quality irrigation water in agricultural production [23]. Rapid urbanization is also forcing agriculture to move into drier or marginal soils [24]. Therefore, although it has been a long-term scientific goal to select crops that can withstand marginal conditions, these efforts have to be intensified under the immediate threat of climate crisis [25–27]. The use of biostimulants in crop production is an innovative and sustainable farming tool that benefits plant growth and crop yield, especially under unfavorable environmental conditions [28,29]. Due to its overall advantages for farmers, the use of biostimulants in field crops is increasingly gaining favor. The composition of sensory important chemicals in aromatic oils is influenced by a variety of agronomic techniques, environmental factors, and cultivar type [30]. Considering the effect of abiotic stressors on secondary metabolites biosynthesis, the use of elicitors as "eustressors" could be beneficial for the production of such compounds and, by extension, of essential oils [31,32].

Seaweed extracts (SWEs) are a new generation of growth stimulants which can also be used as organic fertilizers, thus partially substituting synthetic fertilizers [33–37]. According to several studies, the saccharides in SWEs may trigger plant defense mechanisms [38–40]. The extracts made from the brown alga *Ascophyllum nodosum* (L.) are among the most significant and well-known SWEs so far. Numerous studies have shown that foliar and soil administrations of *A. nodosum* (L.) SWE improved the development of field, fruit and vegetable crops [41–45]. Moreover, arbuscular mycorrhizal fungi (AMF), ectomycorrhizal fungi (ECM) and root-associated plant-growth-promoting rhizobacteria (PGPR) are symbiotic partners that have been shown to benefit plant and soil health in stressful environments [46]. Furthermore, a lot of investigation has been carried out on the effect of different biostimulants' application on crops, such as aromatic and medicinal plants that are grown under abiotic stress conditions, such as water deficit [7,47–50]. In particular, *Ascophyllum nodosum* (L.) extracts were beneficial in oil content, while a significant effect on oil composition and its antibacterial properties was also recorded [51]. Similarly, the foliar application of *Ulva intestinalis* (L.) extracts on hydroponically grown mint plants had positive effects on selected growth and physiological parameters (e.g., leaf fluorescence and maximum quantum efficiency ofPSII (Fv/Fm), leaf relative water content and osmotic potential, electrolyte leakage (%) and photosynthetic pigments, as well as on the water status of plants [52].

In this context, a field experiment was carried out to assess the impact of scheduled deficit irrigation and biostimulant application on yield parameters and water-use efficiency of mint crops. The main goal of this study was to analyze different irrigation scenarios and biostimulants applications in order to maximize production under limited water availability, as well as to increase productivity and essential oil production.

2. Materials and Methods

2.1. Description of Biostimulant Treatments and Experimental Design

The experiment was conducted for two consecutive growing seasons, namely the growing periods of 2021 and 2022. *Mentha arvensis* L. plants were transplanted on 16 May 2021 at the experimental farm of the University of Thessaly, Greece. Each experimental plot was 3.7 m^2, and the plants were arranged in double rows with a plant density of 40.404 plants/ha. The spacing between the centers of the double rows was set at 0.75 m, while the distance between individual plants within each row was 0.33 m. The study included four different biostimulant products, denoted as M1, M2, M3 and M4, as well as a control group (M5) where no biostimulants were added (Figures 1 and 2). The biostimulant products, supplied by Agrology S.A., Greece, consisted of various components, such as vegetable proteins, amino acids, carboxylic acids, seaweed extracts and humic and fulvic acids, as well as calcium, silicon, molybdenum, boron and zinc compounds. More specifically, the first biostimulant, referred to as M1, was a mixture that contained vegetable proteins and amino acids combined with carboxylic acids. The second biostimulant (M2) was a mixture that contained vegetable proteins and amino acids combined with extracts from the seaweed species *Laminaria digitata* (Huds.), Lamouroux and *Ascophyllum nodosum* (L.). The third biostimulatory mixture, denoted as M3, was a balanced solution of humic and fulvic acids combined with extracts from the seaweed species *Laminaria digitata* (Huds.), Lamouroux and *Ascophyllum nodosum* (L.). Lastly, the fourth formulation, designated as M4, contained CaO and SiO_2, along with a Calcium Mobilization and Translocation Factor, as well as trace elements of Mo, Bo and Zn. The detailed composition and the application protocol of the tested biostimulants is presented in Table 1.

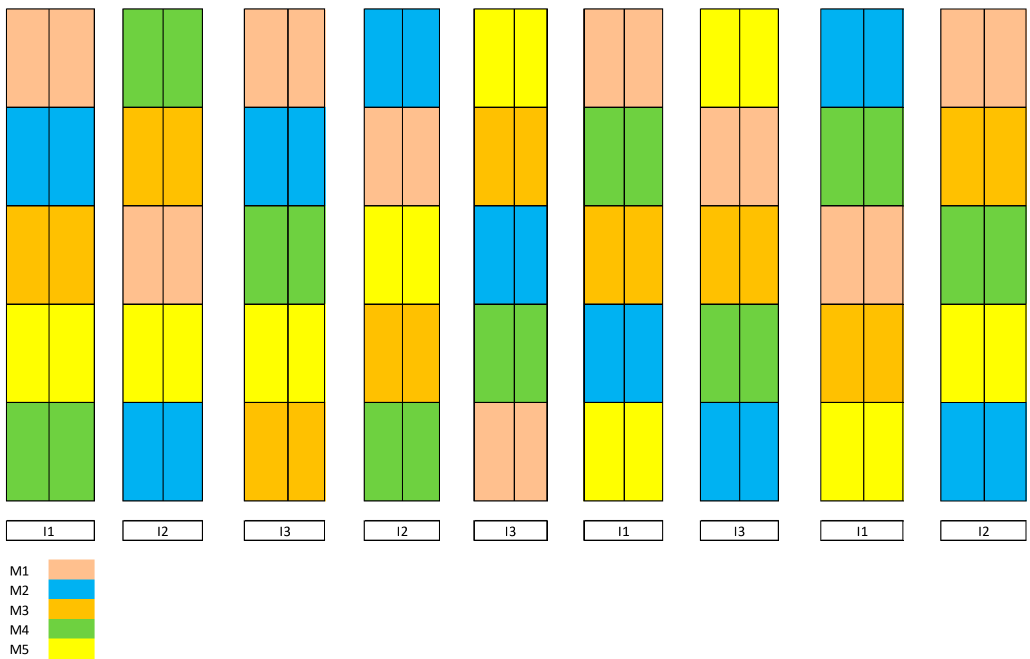


Figure 1. The layout of the experiment: Experimental treatments M1–M5 are described in Table 1. I1 (40% of field capacity), I2 (70% of field capacity) and I3 (100% of field capacity).

Figure 2. Photos of mint plants during the experiment (photos are from the personal record of Dr. Spyridon Petropoulos).

Table 1. Detailed composition and application protocol of the studied biostimulants.

Treatment	Application Method	Composition of Formulation	Application Rate (L/ha)	Number of Applications (Each Growing Season)
M1	Foliar	9.4% free amino acids; 20.6% short chain peptides; 17.1% proteins; 0.7% carboxylic acids	3.5	8
M2	Foliar	2.2% free amino acids; 4.8% short-chain peptides; 4% proteins; seaweed extracts of *Laminaria digitata* (Huds.) Lamouroux (60% of total volume); seaweed extract of *Ascophyllum nodosum* (L.) (20% of total volume)	2	8
M3	Fertigation	Humic and fulvic acids (50% of total volume); seaweed extracts of *Laminaria digitata* (33% of total volume); seaweed extract of *Ascophyllum nodosum* (L.) (17% of total volume)	24	8
M4	Fertigation	27% (*w*/*v*) CaO + 27% (*w*/*v*) SiO_2; calcium mobilization and translocation factor (9.2% of total volume); 0.14 (*w*/*v*) Mo + 2.04% (*w*/*v*) B + 4.1% (*w*/*v*) Zn	11	8
M5	Foliar	Water	-	8

CaO: calcium oxide; SiO_2: silicon oxide; *w*/*v*: weight per volume; Mo: molybdenum; B: boron; Zn: zinc.

The application of biostimulants was performed every 10 days, adding up to 8 times throughout each growing season. M1 and M2 treatments were administered through foliar spraying, while M3 and M4 were applied via fertigation (soil application). Plants treated with M5 (control treatment) received tap water via foliar application until runoff. For foliar spraying, plants were sprayed until runoff. All treatments were applied between 09:00 am to 01:00 pm. Three harvests were conducted, with the 1st occurring in September 2021 (1st growing season), followed by the 2nd in July 2022 (2nd growing season) and the final one in September 2022 (2nd growing season).

2.2. Irrigation Treatments

Three distinct levels of sustained deficit irrigation were also administered and combined with biostimulant treatments. Each irrigation treatment was replicated three times (n = 3). Plants were irrigated via a drip irrigation system with one dripline per row of plants and emitters at a distance of 0.33 cm (one emitter allocated to each plant, namely

40.404 emitters per ha), while the supply of each emitter was 4 L per hour. All plants received the same volume of water from crop establishment till the initiation of deficit irrigation via the irrigation system, namely 288.2 m^3 per ha. Deficit irrigation started approximately 2 months after plant establishment (20 July 2021) with three distinct levels of irrigation, denoted as I1 (40% of field capacity), I2 (70% of field capacity) and I3 (100% of field capacity). Plants were not irrigated after the 1st harvest until April 2022. At that point, all plants received the same amount of water until the initiation of deficit irrigation for the 2nd growing period, namely 97 m^3 per ha. Deficit irrigation started on 2 June until the 3rd harvest, and the same levels of irrigation (e.g., I1-I3) were applied on the same plants as in the 1st growing period.

Soil moisture content was recorded with PR2 Profile Probe (Delta T PR2/4 + HH2; Delta-T devices Ltd., Burwell, UK) using 40 cm long access tubes, while measurements were taken at soil depths of 10, 20, 30 and 40 cm. Soil properties were the following: 48% sand; 29% silt; 23% clay; 1.3%; organic matter; pH 7.9; EC: 1.4 mS/cm; NO_3^-: 9.49 mg/kg; P: 74.53 mg/kg; K_{exch}: 0.98 cmolc/kg; Ca_{exch}: 13.96 $cmol_c$/kg; and Mg: 4.32 $cmol_c$/kg. Access tubes were established with Delta-T augering and extraction kit (PR-ASK1-S, Delta-T Devices Ltd., Cambridge, UK), using one access tube per experimental plot (15 tubes in total). Irrigation was scheduled based on the recordings of soil moisture content taken at regular intervals. Data regarding weather condition (mean air temperature and precipitation were obtained from a field weather station (Wireless Vantage Pro2 Plus, Davis Instruments Corp., Hayward, CA).

The precipitation (mm) and mean air temperature (°C) throughout the growing period are presented in Figure 3. The total amount of precipitation throughout the experimental period was 614.8 mm, which was distributed as follows: 97.4 mm from crop establishment to the 1st harvest; 448.7 mm from the first to the 2nd harvest; and 68.7 mm from the 2nd to the 3rd harvest. The total amount of water that plants received throughout the experiment is presented in Table 2.

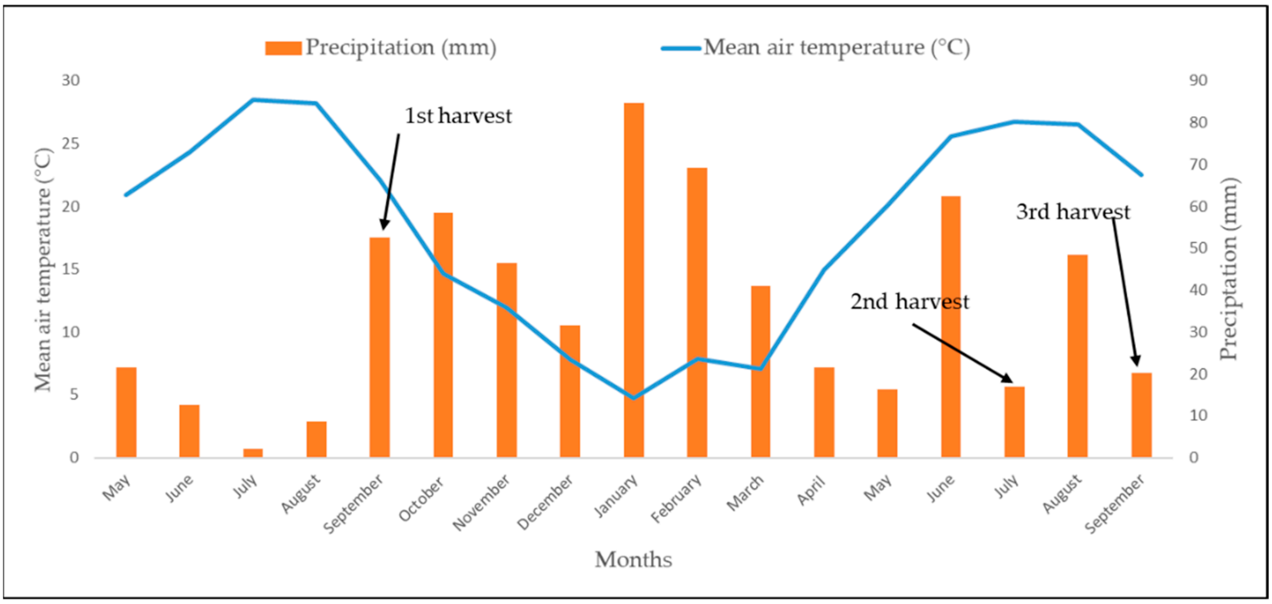


Figure 3. Precipitation (mm of rain) and mean air temperature (°C) throughout the growing period of May 2021–September 2022.

Table 2. The total amount of water (m^3 per ha) that plants received through irrigation during the experimental period.

	Irrigation Water (m^3 per ha)			
	1st Growing Period		2nd Growing Period	
Treatment *	May–July 2021	July–September 2021	April–May 2022	June–September 2022
I1	288.2	206.0	97	159.4
I2	288.2	362.0	97	279.0
I3	288.2	517.2	97	398.6

* I1 (40% of field capacity), I2 (70% of field capacity) and I3 (100% of field capacity).

2.3. Assessment of Crop Performance and Essential Yield

During the harvest period, the assessment of yield parameters was conducted based on measurements of the total fresh weight and dry weight of biomass for each plot. Dry weight was determined with forced-air drying at 65 °C until constant weight. Additionally, a representative sample from each plot was obtained for air-drying at a temperature of 40 °C to evaluate essential oil content and yield. Dried samples were used for the extraction of essential oils and the determination of the essential oil content and yield using a Clevenger apparatus. Briefly, hydrodistillation was performed in 1000 mL flasks using 20 g of dried material and 400 mL of water. Flasks were put in heating mantles (Fibroman-C, Isolab Laborgeräte GmbH, Eschau, Germany) as heating source to facilitate the boiling of distillation water. Each distillation was performed for 2 h, starting from boiling initiation, while each sample was distilled in triplicate (n = 3). After distillation, essential oils were obtained with the use of micropipette to determine the total volume, and then they were put in amber vials under freezing conditions. Essential oil content was expressed as percentage of oil (%; v/w) on a dry weight basis. Essential oil yield per hectare was calculated by multiplying the essential oil content with the dry biomass yield for the respective treatments.

Water-use efficiency (WUE) was calculated based on the following formula [53]:

$$\mathrm{WUE} = \frac{\text{Fresh Yield (kg/ha)}}{\text{Cumulative water supply (mm or m}^3\text{/ha)}}$$

2.4. Statistical Analysis

The experiment was laid out according to split-plot design with three replications per treatment. Irrigation treatment was the main plot, while the biostimulant treatments were arranged as subplots. Each replication included 15 plants, and the whole experiment consisted of 675 plants. For the statistical analysis of data, the two-way analysis of variance (two-way ANOVA) was performed with GenStat (7th Edition) software (VSN International, Hemel Hempstead, UK). For the comparison of means, the least significance difference (LSD) and Tukey's honest significance (HSD) test at $p < 0.05$ were employed.

3. Results

3.1. Growth Parameters—Fresh and Dry Yield

Average fresh and dry biomass (one harvest during the first growing period of 2021 and two harvests during the second growing period of 2022) are presented in Table 3.

The irrigation factor had a significant effect on fresh and dry yield for all the harvests (Table 3). The harvested fresh yield varied between 11,137; 12,730 and 14,652 kg/ha in 2021 for the studied irrigation treatments (I1, I2 and I3), respectively. On the other hand, mint total yield in 2022 (the sum of two harvests), which refers to the second growing season after crop establishment, showed an increase of up to 22,247; 27,129; and 27,583 kg/ha for the I1, I2 and I3 treatments, respectively. It should be mentioned that normally irrigated plants (I3 treatment) performed better only in the case of the first harvest of the first growing period, whereas in the successive harvests, no significant differences between I3 and I2 treatments were recorded in terms of fresh yield. On the other hand, dry fresh yield did not differ between I2 and I3 treatments for all the harvests. Finally, I1 treatment recorded the lowest fresh and dry yield values for all the harvests.

Table 3. The effect of irrigation regime and biostimulant application on crop performance of mint crop (mean ± SD; n = 3).

	Fresh Weight (kg/ha)				Dry Weight (kg/ha)			
Treatments	**1st Harvest 2021**	**2nd Harvest 2022**	**3rd Harvest 2022**	**Total Fresh Weight**	**1st Harvest 2021**	**2nd Harvest 2022**	**3rd Harvest 2022**	**Total Dry Weight**
I1	11,137 ± 237	17,761 ± 588	4486 ± 364	33,384 ± 365	3364 ± 278	4685 ± 631	1354 ± 124	9403 ± 432
I2	12,730 ± 320	20,087 ± 785	7042 ± 521	39,858 ± 846	3665 ± 984	5175 ± 564	1957 ± 523	10,798 ± 777
I3	14,652 ± 435	20,312 ± 256	7271 ± 444	42,235 ± 784	3832 ± 652	5195 ± 788	1950 ± 435	10,977 ± 656
$LSD_{0.05}$	698	1445	868	1810	198	381	228	486
M1	13,344 ± 253	19,562 ± 489	6644 ± 555	39,550 ± 897	3722 ± 384	5077 ± 478	1879 ± 111	10,678 ± 471
M2	14,393 ± 563	20,844 ± 564	6964 ± 476	42,201 ± 974	4168 ± 531	5495 ± 541	1951 ± 89	11,614 ± 521
M3	13,953 ± 355	20,308 ± 893	6811 ± 610	41,072 ± 1042	3965 ± 218	5233 ± 263	1910 ± 186	11,109 ± 431
M4	11,612 ± 654	18,709 ± 745	5691 ± 784	36,011 ± 845	3157 ± 428	4885 ± 401	1661 ± 221	9704 ± 347
M5	10,896 ± 463	17,511 ± 531	5221 ± 563	33,629 ± 931	3090 ± 285	4401 ± 360	1366 ± 106	8857 ± 287
$LSD_{0.05}$	901	1865	1121	2337	256	492	294	627
I1M1	11,786 ± 214	17,848 ± 187	4814 ± 225	34,448 ± 555	3537 ± 231	4722 ± 325	1518 ± 45	9778 ± 103
I1M2	13,348 ± 345	18,841 ± 321	5166 ± 198	37,354 ± 641	4035 ± 198	5151 ± 468	1552 ± 87	10,738 ± 341
I1M3	13,067 ± 361	18,401 ± 784	5089 ± 321	36,557 ± 274	3985 ± 235	4813 ± 169	1535 ± 145	10,333 ± 241
I1M4	9315 ± 254	17,121 ± 564	3750 ± 210	30,186 ± 329	2621 ± 145	4611 ± 340	1191 ± 69	8423 ± 355
I1M5	8171 ± 431	16,595 ± 764	3610 ± 121	28,377 ± 421	2640 ± 89	4129 ± 214	972 ± 54	7741 ± 108
I2M1	13,243 ± 777	20,092 ± 587	7553 ± 224	40,888 ± 354	3758 ± 364	5271 ± 265	2084 ± 156	11,113 ± 402
I2M2	14,147 ± 864	21,977 ± 941	7811 ± 451	43,935 ± 220	4230 ± 410	5672 ± 312	2172 ± 201	12,074 ± 288
I2M3	13,673 ± 745	21,389 ± 871	7604 ± 365	42,666 ± 631	3918 ± 214	5448 ± 178	2110 ± 174	11,476 ± 322
I2M4	11,581 ± 358	19,186 ± 469	6359 ± 258	37,125 ± 521	3276 ± 361	4985 ± 145	1879 ± 101	10,140 ± 198
I2M5	11,005 ± 364	17,789 ± 658	5882 ± 369	34,677 ± 451	3145 ± 215	4497 ± 356	1542 ± 98	9185 ± 235
I3M1	15,003 ± 555	20,746 ± 547	7565 ± 410	43,314 ± 874	3871 ± 315	5236 ± 298	2035 ± 57	11,142 ± 241
I3M2	15,686 ± 456	21,713 ± 654	7916 ± 187	45,314 ± 687	4239 ± 548	5662 ± 187	2130 ± 187	12,031 ± 196
I3M3	15,118 ± 871	21,134 ± 784	7741 ± 291	43,993 ± 631	3992 ± 367	5438 ± 437	2086 ± 141	11,516 ± 158
I3M4	13,939 ± 654	19,820 ± 361	6965 ± 271	40,723 ± 541	3575 ± 421	5060 ± 451	1912 ± 99	10,548 ± 214
I3M5	13,513 ± 543	18,150 ± 521	6170 ± 243	37,832 ± 465	3484 ± 257	4577 ± 324	1585 ± 87	9646 ± 166
$LSD_{0.05}$	ns	ns	ns	ns	ns	ns	ns	ns
CV (%)	7.3	10	18.5	6.3	7.3	10.2	17.4	6.2

Comparison of means was performed with the use of least significant difference (LSD) criterion at $p < 0.05$; I1 (40% of field capacity), I2 (70% of field capacity) and I3 (100% of field capacity); M1–M5: the abbreviations are described in Table 1; CV: coefficient of variation.

A significant effect of the studied biostimulant formulations was also recorded (Table 3). Specifically, the M2 treatment (vegetable proteins combined with seaweed extracts) was the treatment where the highest yield was measured in the first harvest (14,393 kg/ha), while the M1, M2 and M3 treatments were the ones that performed significantly better than the rest of the treatments in the successive harvests of 2022 (Table 1). Moreover, in every case, the control treatment (M5; no biostimulants added) recorded the lowest overall fresh and dry yield. The same trend was observed for total fresh and dry biomass yield, where M2 treatment performed better than the rest of the treatments (42,201 of fresh yield and 11,614 of dry yield), whereas the control treatment (M5) had the lowest overall yield in terms of both fresh and dry weight.

The interaction of the examined factors did not show any statistically significant differences between the various combinations of biostimulant formulation and irrigation regimes (Table 1). However, it should be noted that the I3M2 treatment (full irrigation × M2 biostimulant) was the one with the highest overall yield for all the harvests, whereas the plants that did not receive any biostimulant formulation and were irrigated under the deficit irrigation regime recorded the lowest overall fresh and dry yield. These results are in agreement with the trends recorded for the main effects of biostimulant application and irrigation regime factors.

3.2. Essential Oil Yield

The effect of biostimulant application and deficit irrigation on the essential oil of mint plants is presented in Table 4. The analysis of the results revealed a significant effect of the tested factors, whereas no significant interaction of biostimulant application × irrigation regime was recorded.

Table 4. The effect of irrigation regime and biostimulant application on essential oil content and yield (mean ± SD; *n* = 3).

	Essential Oil Content (%)			Essential Oil Yield (L/ha)			
	1st Harvest 2021	**2nd Harvest 2022**	**3rd Harvest 2022**	**1st Harvest 2021**	**2nd Harvest 2022**	**3rd Harvest 2022**	**Total Essential Oil Yield (L/ha)**
I1	0.854 ± 0.055	1.160 ± 0.101	0.877 ± 0.089	29.39 ± 1.24	54.4 ± 6.66	11.8 ± 1.25	95.6 ± 7.03
I2	0.927 ± 0.087	1.583 ± 0.093	0.768 ± 0.048	34.20 ± 2.18	81.5 ± 4.56	14.95 ± 2.71	130.7 ± 10.69
I3	0.843 ± 0.061	1.114 ± 0.071	0.783 ± 0.035	32.39 ± 3.15	58.0 ± 2.89	15.21 ± 3.01	105.6 ± 5.45
$LSD_{0.05}$	0.059	0.121	ns	2.51	8.4	2.69	9.9
M1	0.852 ± 0.091	1.152 ± 0.091	0.756 ± 0.141	31.68 ± 2.34	58.4 ± 2.96	14.17 ± 1.20	104.3 ± 4.8
M2	1.020 ± 0.058	1.413 ± 0.087	0.824 ± 0.086	42.49 ± 3.31	78 ± 4.12	16.03 ± 2.04	136.6 ± 5.6
M3	0.891 ± 0.074	1.257 ± 0.063	0.800 ± 0.108	35.33 ± 1.98	65.8 ± 3.65	15.11 ± 1.86	116.2 ± 2.9
M4	0.874 ± 0.033	1.209 ± 0.048	0.776 ± 0.159	27.50 ± 2.20	59.3 ± 3.44	12.67 ± 0.89	99.5 ± 4.1
M5	0.737 ± 0.047	1.398 ± 0.055	0.891 ± 0.097	22.96 ± 1.31	61.7 ± 4.12	11.95 ± 1.69	96.6 ± 3.2
$LSD_{0.05}$	0.078	0.156	ns	3.24	10.8	ns	12.8
I1M1	0.822 ± 0.044	1.128 ± 0.076	0.817 ± 0.056	29.08 ± 1.31	53.6 ± 2.31	12.37 ± 0.85	95.1 ± 1.36
I1M2	1.078 ± 0.063	1.194 ± 0.061	0.889 ± 0.038	43.47 ± 0.98	61.5 ± 1.69	13.93 ± 0.69	118.8 ± 2.54
I1M3	0.878 ± 0.078	1.150 ± 0.058	0.878 ± 0.047	35.10 ± 2.31	55.3 ± 3.20	13.40 ± 1.11	103.8 ± 2.65
I1M4	0.867 ± 0.055	1.100 ± 0.079	0.833 ± 0.059	22.72 ± 1.58	50.9 ± 1.54	10.03 ± 0.93	83.6 ± 1.69
I1M5	0.628 ± 0.067	1.228 ± 0.097	0.967 ± 0.088	16.60 ± 1.47	50.7 ± 1.63	9.28 ± 0.68	76.6 ± 1.37
I2M1	0.911 ± 0.088	1.300 ± 0.104	0.706 ± 0.067	34.22 ± 1.03	67.8 ± 1.47	14.94 ± 0.54	117.0 ± 4.56
I2M2	1.033 ± 0.091	1.689 ± 0.89	0.778 ± 0.084	43.71 ± 2.30	95.9 ± 3.24	17.07 ± 0.74	156.7 ± 5.55
I2M3	0.956 ± 0.081	1.594 ± 0.131	0.739 ± 0.039	37.45 ± 3.01	86.3 ± 2.56	15.62 ± 0.93	139.3 ± 5.16
I2M4	0.928 ± 0.069	1.506 ± 0.097	0.722 ± 0.041	30.31 ± 1.25	75.4 ± 4.31	13.45 ± 0.71	119.1 ± 3.69
I2M5	0.806 ± 0.077	1.828 ± 0.124	0.894 ± 0.053	25.31 ± 1.47	82.3 ± 2.68	13.69 ± 0.58	121.3 ± 3.54
I3M1	0.822 ± 0.048	1.028 ± 0.058	0.744 ± 0.039	31.74 ± 2.61	53.9 ± 1.54	15.20 ± 0.35	100.8 ± 4.21
I3M2	0.950 ± 0.092	1.356 ± 0.108	0.806 ± 0.071	40.29 ± 1.88	76.8 ± 2.36	17.10 ± 1.02	134.2 ± 3.25
I3M3	0.839 ± 0.063	1.028 ± 0.055	0.783 ± 0.049	33.45 ± 1.45	55.8 ± 2.54	16.32 ± 0.57	105.5 ± 2.25
I3M4	0.828 ± 0.077	1.022 ± 0.076	0.772 ± 0.039	29.48 ± 1.06	51.7 ± 3.24	14.55 ± 0.69	95.7 ± 1.89
I3M5	0.778 ± 0.083	1.139 ± 0.093	0.811 ± 0.048	26.96 ± 1.55	52.1 ± 4.57	12.88 ± 0.73	91.9 ± 2.22
$LSD_{0.05}$	ns	ns	ns	ns	ns	ns	ns
CV (%)	9.1	12.6	20.5	10.5	17.3	25.7	11.9

Comparison of means was performed with the use of least significant difference (LSD) criterion at $p < 0.05$; I1 (40% of field capacity), I2 (70% of field capacity) and I3 (100% of field capacity); M1–M5: the abbreviations are described in Table 1; CV: coefficient of variation.

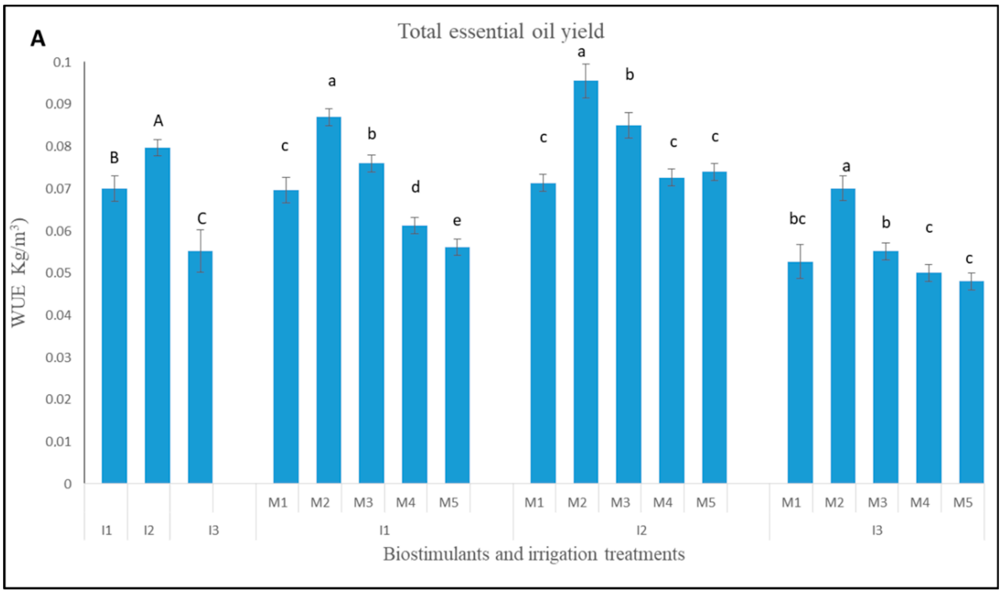
The irrigation factor had a significant effect on essential oil content in the first and second harvest (Table 4). In particular, I2 was the treatment with the highest essential oil content (0.927% and 1.583% in the first and second harvest, respectively), whereas the lowest level of deficit irrigation (e.g., I1 treatment) resulted in the highest essential oil yield without being significantly different from the rest of the treatments. Moreover, normal irrigation resulted consistently in the lowest overall essential oil yield in the first two harvests, while I2 treatment recorded the lowest yield in the third harvest. These findings suggest that moderate water stress could be beneficial to the essential oil biosynthesis and increase the essential oil content of mint plants.

Regarding the tested biostimulant formulations, significant differences between the applied treatments were also observed. M2 was the treatment with the highest essential oil content in the first and second harvest, while in the third harvest M5 treatment recorded the highest overall value. Moreover, M2 treatment differed from the rest of the treatments in the first harvest, whereas no differences were recorded between M2, M3 and the control treatment (M5) in the second one. Finally, the interaction of the examined factors did not show any statistically significant differences between the studied treatments.

The results of the essential oil yield per hectare show that moderate water deprivation (I2 treatment) recorded the highest yield in the first two harvests and the total yield per harvested area, while normal irrigation was the treatment with the highest yield in the third harvest. Moreover, I1 treatments consistently resulted in the lowest essential yield in the successive harvests and consequently in the lowest total yield per hectare. Similar to the essential oil content, the yield of essential oil per harvested area was the highest for the M2 treatment for all the individual harvests and for the total yield, except for the third harvest, where no significant differences were recorded between the tested biostimulant formulations. However, despite the significant impact of the tested factors, no significant interaction between them was recorded for essential oil yield.

3.3. Water-Use Efficiency

The results related to water-use efficiency of mint crop are presented in Figure 4A–C. Despite the amounts of water that plants received through precipitation throughout the growing period and the fact that plants were irrigated until crop establishment in both growing seasons, significant differences were recorded between the tested irrigation regimes.



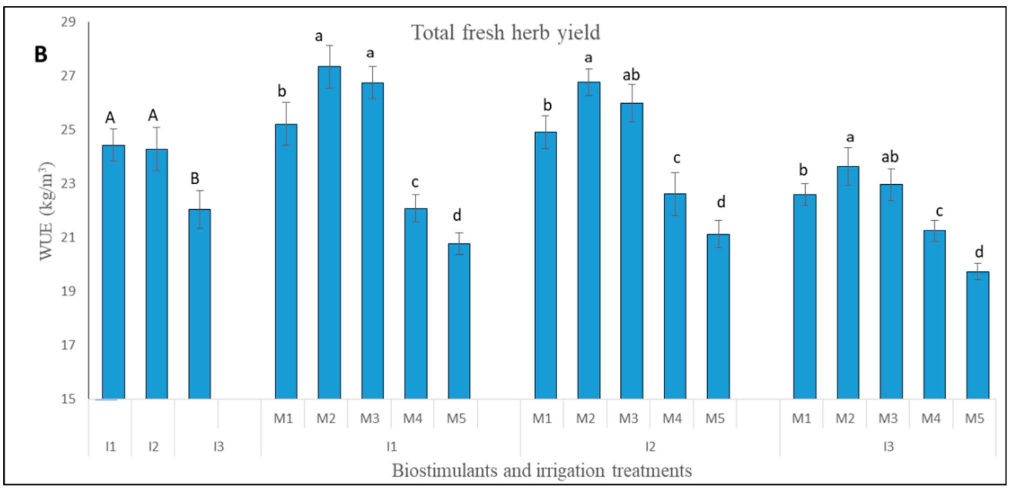


Figure 4. *Cont.*

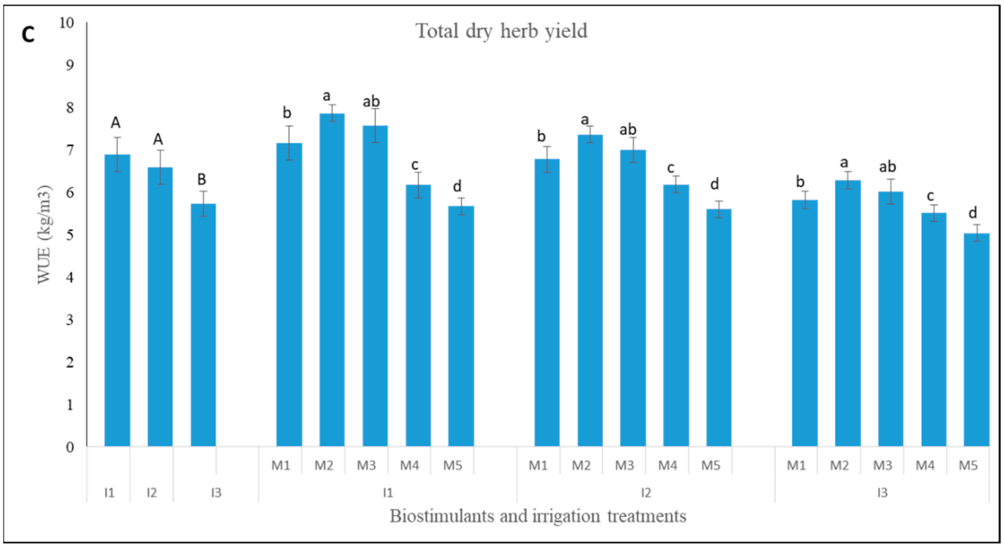


Figure 4. Water-use efficiency (WUE; mean ± SD) of mint plants in relation to biostimulants and irrigation regime based on total essential oil yield (mean ± SD) (**A**); total fresh herb yield (mean ± SD) (**B**); total dry herb yield (mean ± SD) (**C**). Capital letters above bars indicate significant differences between the irrigation treatments, according to Tukey's HSD test at $p = 0.05$. Lowercase letters above bars indicate significant differences between the means of biostimulant treatments for the same irrigation regime, according to Tukey's HSD test at $p = 0.05$. I1: 40% of field capacity, I2: 70% of field capacity; and I3: 100% of field capacity. For biostimulant treatment (M1–M5) descriptions refer to Table 1.

For all the tested yield parameters (e.g., total fresh and dry herb yield and total essential oil yield), deficit irrigation treatments resulted in higher WUE values than normal irrigation, while for total essential oil yield, the WUE values of moderate deficit irrigation (e.g., I2 treatment) were significantly higher than the I1 and I3 treatments (Figure 4A–C). Moreover, the biostimulant application also had a significant impact and significantly increased WUE values compared with the control treatment (M5; no biostimulants), while the M2 and M3 treatments recorded consistently higher values than the rest of the biostimulant formulations and the control treatment (M5; no biostimulants) (Figure 4A–C).

4. Discussion

Modern agriculture has to cope with several restrictions related to climate change and address the issues of replenishment and the sustainable use of natural resources. In the present study, simple agronomic tools such as deficit irrigation and biostimulant application were evaluated as effective practices to mitigate irrigation water shortage in mint crops, focusing on yield parameters and the efficiency of water use.

The tested irrigation treatments had a significant effect on fresh and dry yield for all the harvests, while a significant effect of the studied biostimulant formulations was also recorded. Similarly to our study, Kumar et al. [54] reported similar dry matter yields of approximately 5500 kg/ha, while Zheljazkov et al. [55] and Hanafy et al. [56] also reported that fresh and dry matter yields were lower in the first year and increased from the second year on in *Mentha × piperita*, *Mentha spicata* and *Majorana hortensis* plants. This increase could be due to the better development of rooting systems during the second growing period which facilitated better availability of water and nutrients, especially under water stress conditions [57]. As suggested by Ghamarnia et al. [58], crop evapotranspiration increases within the growing period, which indicates increased water requirements, especially at the end of the growing season. Giannoulis et al. [59] highlighted the effect of environmental conditions (precipitation) on already established lavender plants (*Lavandula angustifolia* L.), which showed a varied yield after six and seven years from crop establishment, while Giannoulis et al. [60] reported similar results for *Salvia officinalis* L. plants for three consecutive growing periods. A gradual increase in yield parameters (fresh and dry biomass and essential oil yield) was also reported for Greek oregano plants (*Origanum vulgare* ssp. *hirtum* (Link) Ietswaart) grown for three consecutive growing periods, where the lowest yields were recorded the first year after crop establishment followed by similar yields the next two years [61]. Moreover, Kumar et al. [54] suggested that simple agronomic practices such as planting method, planting time and planting density may also have a significant impact on the obtained dry yields and improve the use efficiency of resources while reducing cost production at the same time. In the same line, Rama and Kumar [21] suggested fresh herb yield, which was in the same range as in the first harvest of our study (10,600 to 19,300 kg/ha), while the same authors suggested that plant density and nitrogen application may affect herb yield. In the study by Santos et al. [62], a seasonal variation in the fresh and dry herb yield was suggested which highlights the importance of growing conditions on plant growth and crop performance.

Based on our results, the lowest crop production (either fresh or dry) was observed for the I1 irrigation treatment, a finding which is in agreement with the findings of the study conducted by Akbarzadeh et al. [63], who studied the effect of deficit irrigation combined with β-aminobutyric acid application on grapefruit mint and suggested that severe (35% of field capacity) and moderate stress (55% of field capacity) resulted in significantly lower fresh yield per pot.

Finally, it is clearly evident that the treatments with the higher total yield were the ones that contained extracts of various seaweed species combined with either vegetable proteins or with humic and fulvic acids (e.g., M2 and M3, respectively). This finding is consistent with the literature reports, which suggest that biostimulants based on seaweed extracts contain hormones, macro- and micronutrients and saccharides that can significantly improve nutrients uptake and abiotic stress tolerance through better root elongation [51,52,64]. Moreover, the application of seaweed extracts is associated with the induction of bioactive secondary metabolites, which play an important role in plants' defense mechanisms against abiotic stressors [34]. Several other studies that examined the impact of seaweed extracts on plant development, crop production and quality of various crops also support the findings of the present study [65–67]. Although no significant interaction between the tested factors was recorded, it is evident that the combinations of irrigation treatments with biostimulants that contained either vegetable proteins or with humic and fulvic acids showed increasing trends compared with other combinations. Therefore, seaweed extracts could be used as an eco-friendly agricultural practice to increase yield under marginal conditions (e.g., limited water availability).

Essential oil content and oil yield were significantly affected by the tested irrigation and biostimulant treatments. According to the literature, essential oil content and yield of mint crop may vary depending on the growing conditions. In the study by Ram and Kumar [21], essential oil content was in the range of 0.78% to 0.94%, which coincides with the results of our study for the first and third harvest. Moreover, Santos et al. [62] recorded a high variation in essential oil content and yield depending on the season of the harvest and the genotype tested. This variation was also evident in other studies, such as in the reports of Heydari et al. [68], who suggested essential content in the range of 1.84% to 2.63% (*w*/*w*), or the study by Kumar et al. [54], who reported values of 0.42% to 0.82% of essential oil content depending on the plant density, the planting method and the harvesting period, while the respective total oil yield ranged between 129.2 kg/ha and 250.8 kg/ha, which is significantly higher than the yield recorded in our study (68.8 kg/ha to 140.7 kg/ha; oil density of 0.898 g/mL). According to Ramesh and Singh [69], growing conditions may affect biomass allocation to leaves, stems and flowers which, apart from fresh and dry biomass yield, also have an impact on essential oil production, since higher partitioning to leaves and flowers is associated with higher essential oil content and yield. The duration of water deficit is also important for achieving high yields, since, according to Okwany et al. [70], prolonged stress has a severe impact on fresh biomass yield, even at mild water stress conditions, whereas essential oil yield was more affected under severe water stress. Moreover, Chauhan et al. [71] suggested that seasonal variation in essential oil yield could be associated with the effect of growing conditions on monoterpenoids biosynthesis and consequently on essential oil yield. However, it can be also affected by the genotype, as suggested by Détár et al. [72], who studied the essential oil yield parameters of different varieties of lavender (*Lavandula angustifolia* Mill.) and lavandin (*Lavandula* x *intermedia* Emeric ex Loisel.) over two consecutive years.

These various literature reports highlight the effects of seaweed extracts on the root functions of plants, as well as on root/microbe interactions that may regulate water uptake and improve tolerance against water deficit conditions [33,67]. For example, Elansary et al. [7] reported the positive effect of biostimulants based on seaweed extracts and humic acids on growth and yield parameters of mint (*Mentha longifolia* L.) plants grown under moderate and severe water stress. In the same line, liquid extracts obtained from *Ulva intestinalis* (L.) improved the plant growth, photosynthetic functions and water status of peppermint plants [52]. Additionally, Shahabivand et al. [73] and Pourhadi et al. [74] recorded positive effects for foliar and/or topdressing application of humic substances on *Mentha piperita* L. plants grown under field conditions. The positive role of humic substances on soil health is also well established, since they have positive effects on soil physicochemical characteristics (e.g., texture, structure, water holding capacity, cation exchange capacity, enzymes activity, etc.); they improve plant growth through the induction of hormones biosynthesis and increase nutrients availability through chelation and cotransportation of nutrients to plants [75]. In contrast to our study, Rezaei-Chiyaneh et al. [49] suggested that biostimulants based on amino acids were more effective than those contained humic acids against water deficit stress and significantly improved essential oil yield of savory plants. It seems that apart from biostimulant composition, the application method may also affect the effectiveness of the biostimulatory products, since according to Elansary et al. [51], a varied effect was recorded for seaweed extracts on mint plants depending on the application method (e.g., soil drench or foliar application).

Deficit irrigation improved WUE values for tested yield parameters (e.g., total fresh and dry herb yield and total essential oil yield). Similar to our study, Elansary et al. [7] suggested that biostimulant formulation consisting of seaweed extracts (*Ascophyllum nodosum* L.) and humic acid alleviated the negative effects of moderate and severe water stress on the growth and yield parameters of mint plants. These findings are of great importance, since they indicate that biostimulant application and deficit irrigation may improve the use of natural resources without compromising yield parameters. These findings highlight the importance of scheduled deficit irrigation on plants to avoid any stress during crucial stages, such as crop establishment, since this practice could provide all the benefits from irrigation water saving without compromising crop performance and yield parameters. Moreover, the implementation of these agronomic tools could increase water productivity, especially in the arid and semiarid regions of the Mediterranean basin where high competition for irrigation water is combined with water degradation due to anthropogenic activities [76]. Therefore, it is urgent to employ such measures that improve the water-use efficiency of crops, since apart from climate-change-driven impacts, globalization and socioeconomic changes around the globe are expected to further increase water demands and put under threat the reliability of the water resource system in the near future [77].

5. Conclusions

The present study demonstrated that under moderate deficit irrigation (e.g., 70% of filed capacity), there is a notable increase in the percentage of essential oil content and the total essential oil yield per harvested area compared to both full irrigation and severe deficit irrigation (e.g., 100% and 40% of field capacity, respectively). Moreover, the same irrigation treatment did not compromise the fresh and dry herb yield and consequently resulted in higher water-use efficiency for the crop. On the other hand, biostimulant formulations that consisted of vegetable proteins and seaweed extracts (M2 treatment) or seaweed extracts and humic and fulvic acids (M3 treatment) showed consistently better results in terms of fresh and dry herb yield and essential oil yield parameters compared with the rest of the treatments. In conclusion, moderate deficit irrigation conditions and the use of specific biostimulant products could be useful agronomic practices for a more sustainable use of water resources without compromising the crop performance of mint.

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